

Claims:

1. A binding agent comprising two binding moieties, the binding moieties comprising

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(i) a binding member, capable of binding to a binding partner, and

(ii) the binding partner,

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one of said binding moieties being reversibly masked, whereby two such binding agents do not bind one another.

2. A binding agent according to claim 1 wherein the binding member and binding partner of a single binding agent cannot interact with one another after the binding moiety is unmasked.

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3. A binding agent according to claim 2 wherein the binding partner and binding member are sterically incapable of interacting with one another.

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4. A binding agent according to any one of claims 1 to 3 wherein the binding moiety is reversibly masked by a detachable masking element.

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5. A binding agent according to claim 4 wherein the masking element is coupled to the binding agent via a selectively cleavable group or bond.

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6. A binding agent according to claim 5 wherein the selectively cleavable group or bond is cleavable by irradiation, oxidation, reduction, pH change, or enzymatic cleavage.

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7. A binding agent according to claim 6 wherein the irradiation is UV irradiation.

8. A binding agent according to claim 5 wherein the selectively cleavable group or bond is cleavable by a protease.

9. A binding agent according to claim 8 wherein cleavage of the selectively cleavable group or bond activates a protease activity of the binding agent.

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10. A binding agent according to any one of claims 1 to 3 wherein said binding moiety may be unmasked by a conformational change in the binding agent.

10 11. A binding agent according to any one of claims 1 to 10 wherein the binding moieties are an antibody and its cognate epitope.

15 12. A binding agent according to any one of claims 1 to 10 wherein the binding moieties are avidin or streptavidin and biotin.

13. A binding agent according to any one of claims 1 to 12 further comprising an effector member.

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14. A binding agent according to claim 13 wherein the effector member is a signal generating means.

15. A binding agent according to claim 14 wherein the signal generating means is a label moiety or an enzyme.

25 16. A binding agent according to claim 13 wherein the effector member has a binding functionality.

30 17. A binding agent according to claim 16 wherein the effector member is capable of binding to a target cell type.

18. A binding agent according to claim 17 wherein the effector member is capable of binding to a tumour specific antigen or a 35 parasite antigen.

19. A binding agent according to claim 17 wherein the target cell is a cell of the immune system.

5 20. A binding agent according to claim 19 wherein the effector member is capable of activating said cell of the immune system.

21. A binding agent according to claim 20 wherein the cell is a T cell.

10 22. A binding agent according to claim 22 wherein the effector member is an anti-CD3 antibody.

23. A binding agent according to claim 13 wherein the effector member is an enzyme.

15 24. A binding agent according to claim 23 wherein the enzyme is capable of converting a prodrug to a active form.

20 25. A binding agent according to any one of claims 13 to 24 wherein the effector member is reversibly masked.

26. A binding agent according to any one of claims 13 to 25 wherein the effector member is unmasked by the same means as the binding member or partner.

25 27. A binding agent according to any one of claims 13 to 26 comprising at least two different effector members.

30 28. A binding agent according to any one of claims 1 to 27 for use in a method of medical treatment.

29. Use of a binding agent according to any one of claims 1 to 27 in the preparation of a medicament for the treatment of cancer.

35 30. A method of making a binding agent comprising the steps of:

(i) providing a first component comprising a binding member, capable of binding to a binding partner; and

(ii) providing a second component comprising said binding

5 partner,

wherein one of said binding member and binding partner is reversibly masked such that said binding member or partner is prevented from binding to the other; and

10 iii) contacting said first component with said second component such that they become associated with one another.

31. A method according to claim 30 wherein the first and second components are covalently linked to one another.

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32. A method according to claim 30 wherein the first and second components are non-covalently associated with one another.

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33. A method according to claim 32 wherein the first and second components respectively comprise avidin/streptavidin and biotin.

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34. A composition comprising at least two populations of binding agents, each population having at least two binding members for respective binding partners, each of said binding partners being present on another of said populations of binding agents, wherein a binding member of at least one of said populations is reversibly masked, whereby said one population does not bind the population carrying the respective binding partner.

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35. A method of causing aggregation of a plurality of binding agents, comprising

providing a composition comprising at least two populations of binding agents, each population having at least two binding members for respective binding partners, each of said binding partners being present on another of said populations of binding agents, wherein a binding member of at least one of said

populations is reversibly masked, whereby said one population does not bind the population carrying the respective binding partner,

5 and unmasking said binding member, whereby said one population becomes capable of binding the population carrying the respective binding partner.

10 36. A kit comprising at least two populations of binding agents, each population having at least two binding members for respective binding partners, each of said binding partners being present on another of said populations of binding agents, wherein a binding member of at least one of said populations is reversibly masked, whereby said one population does not bind the 15 population carrying the respective binding partner.

37. A method of determining an analyte in a sample, the method comprising

20 (a) contacting the sample with a plurality of binding agents each comprising two binding moieties, the binding moieties comprising

25 (i) a binding member, capable of binding to a binding partner, and

(ii) the binding partner,

one of said binding moieties being reversibly masked, whereby said binding agents do not bind one another;

30 (b) contacting the sample with a detecting agent capable of binding to the analyte if present, wherein the detecting agent comprises one of said binding moieties; and

35 (c) unmasking said reversibly masked binding moiety, whereby said binding agents form an aggregate.

38. A method according to claim 37 wherein the detecting agent is one of the binding agents.

5 39. A method according to claim 37 or claim 38 comprising the step of detecting the presence of aggregated binding agents.

40. A method according to claim 39 wherein the binding agents comprise signal generating means.

10 41. A method according to any one of claims 37 to 40 wherein the analyte is immobilised on a solid phase.

15 42. A method according to any one of claims 37 to 41 wherein the analyte is a protein, polypeptide, peptide, carbohydrate, nucleic acid, organic or inorganic polymer, or a small organic or inorganic molecule.

43. A method according to any one of claims 37 to 42 wherein the detecting agent is an antibody specific for the analyte.

20 44. A method according to claim 43 which is an ELISA, Western blot, immunohistochemistry or immunofluorescence assay.

25 45. A method according to any one of claims 37 to 42 wherein the detecting agent is a nucleic acid molecule capable of hybridising to the analyte.

46. A method according to claim 45 which is a Southern blot, Northern blot or in situ hybridisation.

30 47. A method of determining an analyte in a sample, the method comprising

(a) contacting the sample with

35 (i) a competitor, and

(ii) a plurality of binding sites, wherein each binding site is capable of binding to the analyte if present and to the competitor, but not to both simultaneously;

5 (b) contacting the sample with a plurality of binding agents each comprising two binding moieties, the binding moieties comprising

(i) a binding member, capable of binding to a binding partner, and

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(ii) the binding partner,

one of said binding moieties being reversibly masked, whereby said binding agents do not bind one another; and

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(c) contacting the sample with a detecting agent capable of binding to the competitor if present, wherein the detecting agent comprises one of said binding moieties; and

20 (d) unmasking said reversibly masked binding moiety, whereby said binding agents form an aggregate.

48. A method of determining an analyte in a sample, the method comprising

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(a) contacting the sample with at least two populations of binding agents, each population having at least two binding members for respective binding partners, each of said binding partners being present on another of said

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populations of binding agents, wherein a binding member of at least one of said populations is reversibly masked, whereby said one population does not bind the population carrying the respective binding partner, and

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(b) contacting the sample with a detecting agent capable of binding to the analyte if present, wherein the detecting agent comprises one of said binding members or binding partners,

(c) unmasking said reversibly masked binding member, wherein said binding agents form an aggregate.

5 49. A method of determining an analyte in a sample, the method comprising

(a) contacting the sample with

10 (i) a competitor, and

(ii) a plurality of binding sites, wherein each binding site is capable of binding to the analyte if present and to the competitor, but not to both simultaneously;

15 (b) contacting the sample with at least two populations of binding agents, each population having at least two binding members for respective binding partners, each of said binding partners being present on another of said populations of binding agents, wherein a binding member of at least one of said populations is reversibly masked, whereby said one population does not bind the population carrying the respective binding partner;

25 (c) contacting the sample with a detecting agent capable of binding to the competitor if present, wherein the detecting agent comprises one of said binding members or binding partners; and

30 (d) unmasking said reversibly masked binding moiety, whereby said binding agents form an aggregate.

50. A method of causing aggregation of a therapeutic agent at a physiological site, the method comprising administering a plurality of binding agents to an individual, each of said binding agents comprising two binding moieties, the binding moieties comprising

(i) a binding member, capable of binding to a binding partner, and

(ii) the binding partner,

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one of said binding moieties being reversibly masked, whereby said binding agents do not bind one another, each of said binding agents further comprising said therapeutic agent,

10 and wherein the method further comprises the step of inducing aggregation of said binding agents by unmasking said reversibly masked binding moiety such that said binding agents bind one another.

15 51. A method according to claim 50 wherein the physiological site is a disease site.

52. A method according to claim 51 wherein the disease site is a tumour or a site of parasite infection.

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53. A method according to claim 50 wherein the physiological site is the site of a wound.

25 54. A method according to any one of claims 50 to 53 comprising administering a targeting agent capable of binding to a molecule which is expressed at the site and capable of binding to one of the binding agents or capable of unmasking said binding moiety.

30 55. A method according to any one of claims 50 to 54 wherein the binding moiety is unmasked by illumination or enzyme action.

35 56. A method according to any one of claims 50 to 55 wherein the therapeutic agent is capable of binding to a molecule expressed on a cell surface, a molecule capable of activating a cell of the immune system, a drug or prodrug molecule, or an enzyme.

57. A method according to claim 56 wherein the therapeutic agent is an anti-CD41 antibody, and anti-CD3 antibody or an enzyme capable of converting a prodrug to an active form.

5 58. A method according to any one of claims 50 to 57 wherein the therapeutic agent is reversibly masked and can be unmasked by the same mechanism as the binding moiety.

10 59. A method of causing aggregation of a therapeutic agent in a culture of cells, the method comprising contacting a culture of cells with a plurality of binding agents, each of said binding agents comprising two binding moieties, the binding moieties comprising

15 (i) a binding member, capable of binding to a binding partner, and  
(ii) the binding partner,

20 one of said binding moieties being reversibly masked, whereby said binding agents do not bind one another, each of said binding agents further comprising said therapeutic agent,  
25 and wherein the method further comprises the step of inducing aggregation of said binding agents by unmasking said reversibly masked binding moiety such that said binding agents bind one another.

60. A method according to claim 59 wherein the cells comprise  
30 cancer cells, parasitically-infected cells, cells of the immune system or platelets.